High performance liquid chromatography for direct and indirect enantiomeric separations of chiral drugs

Witte, Dirk Theodoor

II.2: Optimization of the reaction conditions for the derivatization reaction of promethazine with (-) menthylchloroformate.

Introduction

For the indirect separation of the enantiomers of promethazine and similar substances by high performance liquid chromatography derivatization with (-) menthylchloroformate in the presence of triethylamine is a good method.[1,2] The derivatization reaction did not show racemization and results in a demethylation of the tertiary amine and the formation of a urethane. Earlier studies showed that after derivatization with (-) menthylchloroformate the formed diastereoisomers could be separated on a revered phase high performance liquid chromatography system with a resolution of about 2 within 25 minutes.[1] The influence of the chemical structure of the tertiary dimethylamine on resolution and retention time was remarkable.[2] The above results were obtained under a given set of arbitrary chosen conditions for the derivatization reaction.

The present study was undertaken to find out if the reaction conditions could be optimized towards the largest possible yield. This was done studying the shape of the response surface area. The yield of the derivatization product was calculated through a summation of the areas from the chromatographic peaks of the diastereoisomers. This amount is further regarded as the response. The influence of the variables temperature (Temp), reaction time (Time) and the percentage of triethylamine in the reaction mixture (Tea) on the response (Res) was studied. The variables and their settings in the heart of the design were based on experience from earlier studies.[1] Techniques used for the optimization were Response Surface Methodology in combination with a Factorial Design.[3] Statistic polynomial models were used to describe the effects of the variables on the response within the factor space covered by the design. In theory, models to describe the effects may contain linear and/or quadratic terms and maybe even terms of higher order. Effects caused by interactions between the variables were also taken into account. With the thus obtained model the optimal reaction conditions for the derivatization reaction were calculated. In the heart of the design several measurements were made on different days to calculate the reproducibility in the response.

Experimental

-Apparatus

The RP-HPLC system contained a (150 *x* 4.6 mm, i.d.) stainless steel column, filled with Nucleosil C_{18} 5 μm (Macherey, Nagel, Dueren, Germany). The solvent delivery system was a 2150 LKB HPLC pump (Pharmacia LKB Biotechnology, Uppsala, Sweden), 20 μl injections were made by a 710 A WISP (Waters Associates Inc, Milford, MA, USA) and detection was carried out by a Model 770 spectrophotometric detector at 254 nm (Spectra-Physics, Santa Clara, CA, USA). For integrating the chromatograms a C-R3A Chromatopac (Shimadzu, Kyoto, Japan) was used. The models describing the influences of the variables on the response were calculated by means of the SAS 6.04 package (SAS Institute, Cary, NC, USA). The mobile phase used in all experiments consisted of acetonitrile / 0.1 M aqueous acetic acid pH 2.9, 75/25 (v/v) both separately filtered through a 0.45 μm membrane filter (Schleicher & Schüll, Dassel, Germany). Mixtures were prepared by volume and degassed in a Sonicor (Farmingdale, NY, USA) ultrasonic bath before using the eluent. The flow rate was 1.0 ml/min.

-Chemicals

Racemic promethazine.HCl was obtained from Brocacef (Maarssen, The Netherlands) and was of Ph. Eur. quality. Acetonitrile was from Westburg (Leusden, The Netherlands) and was HPLC grade. Triethylamine was purchased from Janssen (Beerse, Belgium) and was of analytical grade. (-) Menthylchloroformate; {$[\alpha]_D^{18}$: -80° (1g/100ml chloroform)}, was obtained from Aldrich-Chemie (Steinheim, Germany). Acetic acid, ether, anhydrous sodium carbonate and sodium hydroxide pellets, all of analytical grade, were from E. Merck (Darmstadt, Germany).

-Extraction of promethazine base

50 mg Promethazine.HCl was dissolved in 5 ml distilled water. A few drops of a 2 M sodium hydroxide solution were added to this solution to adjust the pH to about 10. The obtained solution was extracted with 5 ml ether by shaking the mixture vigorously. The ether layer was separated. The water layer was extracted two more times with 5 ml of ether. The combined ether layers, dried with solid anhydrous sodium carbonate, were evaporated under a gentle stream of nitrogen at room temperature. The resulting promethazine base was used in the derivatization reactions. All handlings with

promethazine were done in subdued light to prevent decomposition.

-The settings of the reaction conditions used during the optimization

Three solutions of promethazine, A,B and C, of 41.3 μg/ml each, in acetonitrile/triethylamine were prepared. The amount of triethylamine in these solution differed, A:0.83%, B:9.09% and C:17.35% (v/v). For derivatization, 100 μl A-C was mixed with 250 μl acetonitrile/(-) menthylchloroformate 90:10 (v/v) in a conical WISP vial (Waters Associates Inc. Milford, MA, USA) of 400 μl. Each vial was placed in a larger WISP vial of 4 ml which was sealed with a Teflon seal. The sealed vials were placed in an oil bath containing Min 200 mineral oil (Haake, Karlsruhe, Germany) which was heated by a Thermomix 1442 D thermostat (Braun, Rijswijk, The Netherlands). To stop the reaction the sealed vials were placed in ice. The settings of the variables studied with the corresponding scaled values are given in Table 1.

Table 1: The settings of the variables studied together with the scaled values (-1,0,+1)

Time (min)		Temperature (°C)		% Triethylamine (v/v)	
30	- 1	30	- 1	0.83	- 1
90	0	55	0	9.09	0
150	+ 1	80	+ 1	17.35	+ 1

The model used to describe the effect of the variables on the response was based on the scaled values for the variables. The conditions for each experiment are given in Table 2. Experiments 1-12 were done on day 1 and experiments 13-21 on day 2. The heart of the design (0,0,0) was measured several times on both days to study the reproducibility of the method and the possibility of the occurrence a block effect between days 1 and 2.

Results and discussion

After the experiments on day 1 there were reasons to believe that the variables had quadratic effects on the response. The mean response from experiments 1-8 was significantly different, at a 95% confidence interval, from the mean response in the heart of the design, experiments 9-12. To be able to model quadratic effects, the data set had

to be extended with the experiments from day 2. To study the possibility of a block effect between day 1 and 2 the heart of the design was measured three times, experiments 19-21, on day 2 again. There was no significant difference between the mean values in the heart of the design on days 1 and 2 respectively, so that the data from day 1 could be correlated with the data from day 2.

Table 2: The scaled reaction conditions for each experiment

Experiment nr	Time	Temperature	Triethylamine
1	- 1	- 1	- 1
2	+ 1	- 1	- 1
3	- 1	+ 1	- 1
4	+ 1	+ 1	- 1
5	- 1	- 1	+ 1
6	+ 1	- 1	+ 1
7	- 1	+ 1	+ 1
8	+ 1	+ 1	+ 1
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	+ 1	0	0
14	0	+ 1	0
15	0	0	+ 1
16	- 1	0	0
17	0	- 1	0
18	0	0	- 1
19	0	0	0
20	0	0	0
21	0	0	0

With these experiments, and the resulting data set, the model to describe the effects of the variables on the response could be calculated with SAS. The resulting model is given in Equation 1.

Equation 1: The model describing the influence of temperature (Temp), time and % triethylamine (Tea) on the response (Res)

$$\text{Res} = 26625 + 1436\,x\,\text{Time} + 2248\,x\,\text{Temp} - 3593\,x\,\text{Tea} - 1259\,x\,\text{Time}\,x\,\text{Tea} + 1540\,x\,\text{Time}\,x\,\text{Temp}\,x\,\text{Tea} - 3000\,x\,\text{Time}\,x\,\text{Time} - 6908\,x\,\text{Temp}\,x\,\text{Temp} - 2749\,x\,\text{Tea}\,x\,\text{Tea}$$

The R^2_{adj} was 0.90 which shows that 90 % of the variance in the response is described by the model.[4] $R^2_{adj}=1$ if all the variance in the response is explained by the model. If the variance in the response cannot be explained by the model this R^2_{adj} becomes smaller.

Equation 1 was obtained by fitting all possible combinations of variables, quadratic effects and interactions, until the combination of variables with the highest R^2_{adj} was found. With this model the setting of the variables yielding the largest response was calculated. The optimal scaled values were Time:0.36, Temp:0.14 and Tea:-0.72, respectively. These corresponds to 112 minutes, 59 °C and 3.1 % triethylamine.

Figures 1 and 2 show the surface response areas based on the model given in Equation 1. From Figures 1 and 2 it can be seen that there is a maximum response inside the factor space covered by the design. Figures 1A, B and C give the response in relation to temperature and time for three percentages of triethylamine. The largest response is obtained if the percentage triethylamine equals 3.1.

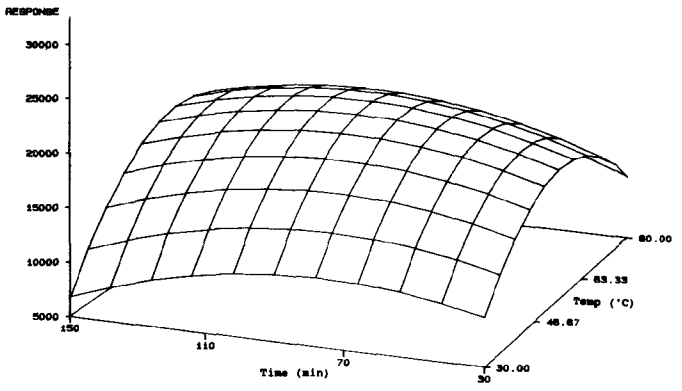

Figure 1A: Response as a function of time and temperature at a percentage triethylamine of 17.35.

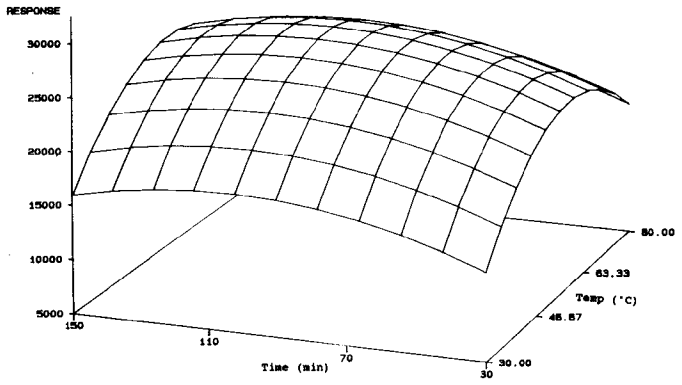

Figure 1B: Response as a function of time and temperature at a percentage triethylamine of 9.09.

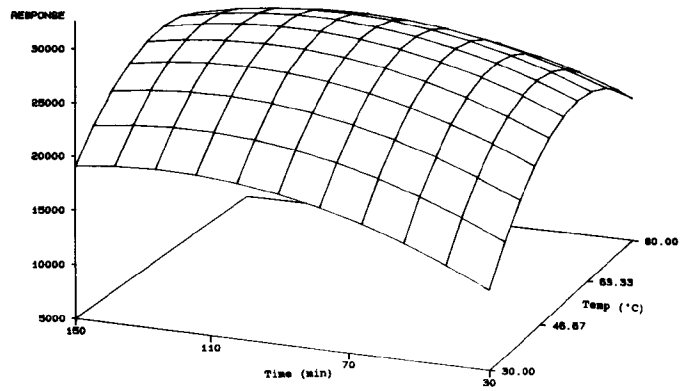

Figure 1C: Response as a function of time and temperature at a percentage of triethylamine of 3.1.

Figure 2 shows the responses measured at the heart of the design in relation to temperature and the percentage triethylamine, Figure 2A, and in relation to time and the percentage triethylamine, Figure 2B.

From Figures 1 and 2 it can be seen that the temperature has the largest effect on the response but that the optimal temperature is not the highest one.

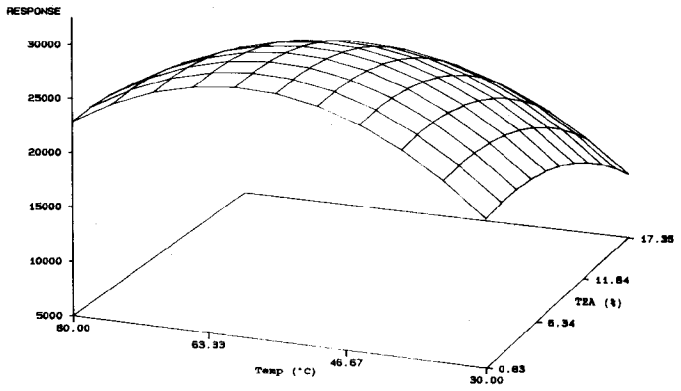

Figure 2A: Response as a function of temperature and percentage triethylamine with a reaction time of 90 minutes.

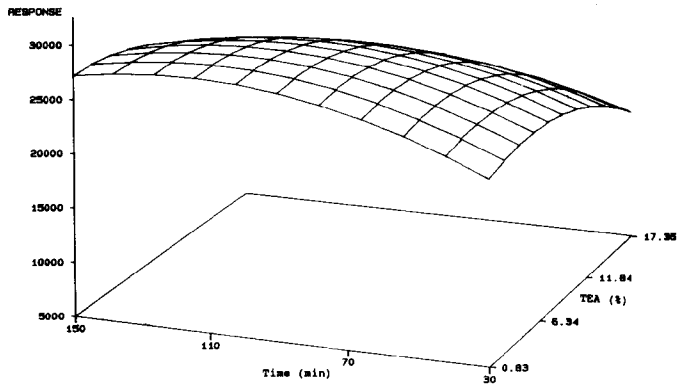

Figure 2B: Response as a function of time and percentage triethylamine at a temperature of 55 °C.

Table 3 gives the responses from the model together with the responses actually measured. The 95 % confidence interval for the predicted response under optimal conditions is 28366 (+/-) 3188, n=4. Finally, four experiments were done under the predicted optimal conditions. The 95 % confidence interval for the mean actually measured response under optimal conditions was 25086 (+/-) 299, n=4. This means that

there is no significant difference between the mean values of the predicted and actually measured responses under optimal conditions. Also there is no significant difference between the response under optimal conditions and the response actually measured in the heart of the design, for the latter the 95 % confidence interval is 25510 (+/-) 514, n=7.

Table 3: The actually measured values together with the theoretically predicted values

Experiment nr.	Pred. response	Meas. response	$\frac{\text{(Meas.- Pred.)}}{\text{(Meas.)}} \times 100$
1	11075	10637	-4.1
2	19547	20974	+6.8
3	18653	19220	+3.0
4	20963	19726	-6.3
5	9487	7305	-29.9
6	6761	6444	-4.9
7	10904	10948	+0.4
8	14339	12578	-14.0
9	26625	25898	-2.8
10	26625	25345	-5.1
11	26625	26465	-0.6
12	26625	25536	-4.3
13	25060	26950	+7.0
14	21965	24354	+9.8
15	20282	24500	+17.2
16	22188	24199	+8.3
17	17467	18979	+8.0
18	27469	27152	-1.2
19	26625	25417	-4.8
20	26625	24695	-7.8
21	26625	25218	-5.6
			-1.5[1]

1:Mean value of: $\frac{\text{(Meas. - Pred.)}}{\text{(Meas.)}} \times 100$

Conclusion

The optimal conditions found were inside the factor space covered by the design and could easily be attained in practice. The slope of the response surface inside the factor space is not rather steep and therefore small differences in the settings of the variables do not have a dramatic effect on the response. Thus the reaction can be considered to be relatively robust. It should be noted that extrapolation of the data from this study to other amines and/or derivatization agents cannot be done. This means that for each combination of an amine with a derivatizing agent the optimal conditions have to be established through experimental studies.

Acknowledgement

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References

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